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Conflicting Morphological and Reproductive Species Boundaries in the Coral Genus *Platygyra*

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Abstract. In mass-spawning corals, potential exists for gametes of a number of species to mix in the water column. The existence of morphologically distinct sympatric coral populations despite such an event implies the presence of isolating mechanisms to prevent hybridization and maintain species boundaries. Over 380 fertilization trials were conducted to determine the extent of reproductive isolation among the seven morphologically defined species (morphospecies) of the scleractinian coral genus *Platygyra*, on the Great Barrier Reef. Results from these experiments demonstrate that fertilization between-morphospecies can occur at rates equivalent to within-morphospecies fertilizations, indicating that no gametic-level barriers to fertilization exist among these morphological species. Observations of spawning times both in the field and in the laboratory have shown that all seven morphospecies spawn on the same night and that there is considerable overlap in the hour of spawning among them. These data indicate that few, if any, temporal barriers to fertilization exist among morphospecies of *Platygyra* on the Great Barrier Reef. In addition, larvae resulting from between-morphospecies crosses are capable of settlement and subsequent growth equivalent to that of within-morphospecies larvae. Our results reveal a discontinuity between reproductive and morphological species boundaries within the scleractinian genus *Platygyra* and challenge species definitions within the Scleractinia. It is not yet clear what mechanisms might maintain morphological boundaries in *Platygyra* in the face of the clear potential for gamete mixing. The disjunct distributions of certain morphospecies along latitudinal and habitat boundaries, and the small levels of variation in reproduction may be two such mechanisms.

Introduction

Species boundaries in scleractinian corals are primarily based on skeletal morphology (*e.g.*, Ellis and Solander, 1786; Vaughan and Wells, 1943; Veron *et al.*, 1977; but see Lang, 1984). It is generally assumed that morphological differences between coral species are highly correlated with reproductive incompatibility (*i.e.*, biological species boundaries) (Willis, 1990) and that traditional theories of hierarchical evolution and speciation (*e.g.*, Darwin, 1859) will apply to corals.

Recognition that most of the corals on the Great Barrier Reef reproduce annually during a mass spawning event (Harrison *et al.*, 1984; Willis *et al.*, 1985; Babcock *et al.*, 1986) has prompted an examination of assumptions that morphological species boundaries reflect biological species boundaries within the Scleractinia (Wallace and Willis, 1994). The persistence of morphologically distinct species groups (*e.g.*, Veron *et al.*, 1977) coupled with mass spawning events, whereby gametes of many species become mixed within the water column, implies the presence of some mechanism to prevent hybridization and hence maintain species boundaries (Willis, 1990). The benefit of such mechanisms was supported by results from experimental crosses in the genus *Montipora*, in which hybridization was documented but considered to be maladaptive due to arrested development in hybrid embryos (Hodgson, 1988). More recently, however, fertilization experiments have indicated that successful fertilization *in vitro* is possible among some morphological coral species (Willis *et al.*, 1992), although how experimental fertilization relates to field fertilization is unknown.

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The coral genus *Platygyra* includes morphological species (morphospecies) from the Great Barrier Reef; *Platygyra daedalea, P. sinensis, P. pini, P. lamellina, P. ryukyuensis, Platygyra* 'B', and *Platygyra* 'H' (see Miller, 1994a, b). These morphological species have been recognized by traditional taxonomy (*e.g.*, Chevalier, 1975; Veron *et al.*, 1977; Veron, 1993) and numerical taxonomy (Miller, 1994a), although both approaches have recognized a continuum of separate characters among the species. Based on the occurrence of morphologically distinct species of *Platygyra*, it seems highly likely that mechanisms exist that promote reproductive incompatibility and subsequently maintain species differences within the genus.

Contrary to expectations, genetic comparisons show no differentiation between the morphological species of *Platygyra* (Miller and Benzie, in press). The high genetic similarity of *Platygyra* morphospecies may reflect recent speciation events following which little genetic divergence has taken place (Miller and Benzie, in press). Alternatively, gene exchange may occur naturally between the morphological species groups, and hence the morphological boundaries in this genus may not necessarily represent reproductive isolation between species. The relationship between reproductive and morphological species boundaries within *Platygyra* and the presence or absence of reproductive isolation between the morphological species therefore need to be established.

A number of mechanisms, including pre- and post-zygotic barriers, could mitigate reproductive isolation between coral species. In *Platygyra*, there is some indication of temporal differences (in the order of hours) in spawning times among some of the morphospecies (Babcock *et al.*, 1986), although these observations involved only a few colonies, and variability in spawning times within morphospecies has not been documented. Coral gametes remain viable for at least 5 h in the water column (Oliver and Babcock, 1992), and it is not known whether these short temporal differences in spawning act as a complete isolating mechanism.

Sperm chemotaxis is used by a variety of marine invertebrates to attract conspecific sperm (*e.g.*, Miller, 1979, 1985), and may well be important in reducing potential for hybridization, for example, in pelagic communities of hydromedusae (Miller, 1979). Chemical sperm attractants have also been implicated as a mechanism to reduce hybridization in the coral genus *Montipora* (Coll *et al.*, 1994). However, even in *Montipora*, where sperm attractants have actually been identified, chemotaxis is not completely specific, and heterospecific attractants may still elicit a degree of chemotactic behavior between species (Coll *et al.*, 1994; Richard Miller, pers. comm.). At this point, the role of sperm chemotaxis as an effective isolating mechanism during mass spawning is still speculative.

Species-specific gamete-binding proteins have been described in some marine invertebrates, including abalone (Vacquier *et al.*, 1990) and sea urchins (Palumbi, 1992; Metz and Palumbi, 1996). Similar proteins may play an important role in the reproductive isolation of many free-spawning invertebrates, including scleractinian corals.

The high predictability of spawning in *Platygyra* species on the Great Barrier Reef (Babcock *et al.*, 1986) makes it possible to explore reproductive relationships and potential isolating mechanisms between these corals at the gamete level. In this paper, we document results from more than 380 trials that show potential for fertilization between the morphological species of *Platygyra*. Parallel field observations of spawning times suggest that few if any temporal reproductive barriers exist between the morphological species. This evidence suggests there are no pre-zygotic isolating mechanisms between the morphological species of *Platygyra*. Furthermore, we found no differences in the ability of larvae from within-morphospecies and between-morphospecies crosses to settle and grow. We also examined the effects of gamete aging, sperm dilution, and parental genotype on fertilization success.

The discovery that successful fertilization can occur between morphological species of *Platygyra* calls into question the previous assumption that morphological species boundaries equate with reproductive isolation and subsequently with evolutionary units. Our results prompt a reexamination of species definitions within the Scleractinia and indicate that species groupings based solely on morphology are clearly not always "biological" species (Mayr, 1942). Our data, combined with other recent findings in this field (Knowlton *et al.*, 1992; Van Veghel and Bak, 1993; Van Veghel, 1994; Wallace and Willis, 1994), suggest that species definitions in the Scleractinia should perhaps be based on a range of criteria, rather than solely on reproductive or morphological evidence. In addition, we suggest that in *Platygyra*, even with genetic exchange, discrete morphological groups will be maintained through a combination of spatial and temporal variability in reproductive processes and fitness in a variety of habitats.

Materials and Methods

We conducted fertilization experiments and observed spawnings over a $3\frac{1}{2}$-year study period during seven consecutive spawnings at three reefs in the central Great Barrier Reef: Magnetic Island—19°10′ S, 146°51′ E (1990, 1991, 1992, 1993); Davies Reef—18°50′ S, 147°39′ E (1990, 1991); and Orpheus Island—18°35′ S, 146°29′ E

(1992). On the afternoon of the predicted mass spawning, gravid *Platygyra* colonies were transferred from the reef into separate containers and kept under natural light to simulate normal conditions. Colonies generally commenced spawning from dusk onwards. Once a colony had spawned, gamete bundles were collected from the surface of the water and sperm and eggs were separated by gentle agitation and use of a 105-μm sieve. Sperm were stored at room temperature (about 27°C) in a concentrated form to reduce aging (see Chia and Bickell, 1983; Oliver and Babcock, 1992) until required for fertilization. Eggs were taken through a series of 8 to 10 washes of 'sperm-free water' (collected half a kilometer from the edge of the reef on the afternoon before the commencement of spawning) to ensure the absence of any trace sperm. Eggs were stored in glass jars with gentle agitation until needed for experiments.

Eggs and sperm from different colonies were combined experimentally in 20-ml glass scintillation vials to examine fertilization success between colonies of different morphologies. Morphological species identification of all colonies used in fertilization trials was based on Miller (1994a). Concentrations of the stored sperm were estimated by five replicate hemacytometer counts and, just before fertilization, diluted to a concentration of approximately 2.5×10^6 sperm/ml. This sperm concentration has been shown to give optimum rates for fertilization in trials with *Platygyra sinensis* and other coral species (Oliver and Babcock, 1992). About 100 eggs were added to 20-ml aliquots of diluted sperm in a vial. Each cross was replicated either two or three times. Sperm concentrations within experimental vials were checked randomly and found to be within 10% of estimated concentrations.

Experiments were designed in a matrix system in which all colonies were crossed reciprocally with all other colonies (eggs A × sperm B and eggs B × sperm A, etc.), although trials between some morphological species have not yet been conducted due to the time constraints associated with coral spawning. All colonies were tested for their ability to self-fertilize (eggs A × sperm A, eggs B × sperm B, etc.). Replicate controls were prepared by placing eggs from each colony in 20 ml of sperm-free water to determine whether there had been any sperm contamination of washed eggs. Glass vials containing eggs and sperm were kept at ambient sea temperature with gentle agitation to allow fertilization to take place.

Fertilization in all replicate vials was recorded after 2–3 hours and again after 6–8 hours by censusing the first 100 eggs sampled from any one vial. Fertilization was scored as the initiation of cleavage, 2–3 h after fertilization (Babcock and Heyward, 1986). After about 7 h most of the embryos were blastulae. The condition of eggs and embryos in all crosses was recorded as either 'regular'—with even, symmetrical cleavages and apparently normal development (see Babcock and Heyward, 1986); or 'irregular'—with uneven, asymmetrical cleavages and abnormal development.

Analysis of fertilization data

Percent fertilization in crosses after 2–3 h and 6–8 h was based on the total number of eggs censused in each vial. Percent fertilization after 6–8 h was calculated to detect any increase in fertilization over egg-sperm contact time. The percentage of fertilized eggs developing to blastulae between the two counts for each vial was calculated as the number fertilized after 6–8 h (including cleavages and embryos) expressed as a percentage of the number of fertilized eggs after 2–3 h. Mean percent fertilization of the three replicate vials in each cross (colony pairing) was used for the analyses. Any crosses for which the controls showed sperm contamination of eggs during the washing process were not used in the analyses. Results from the seven spawnings at three reefs were combined at the level of morphological species for analysis.

A two-way analysis of variance (ANOVA) based on the percent fertilization data after 2–3 h was carried out to determine whether fertilization rates differed either between between-morphospecies and within-morphospecies fertilizations or between the different morphospecies (based on maternal colony type). The main effects were fertilization type (2 treatments; within and between morphospecies) and egg species (7 treatments; *Platygyra* B, *P. daedalea*, *Platygyra* H, *P. lamellina*, *P. pini*, *P. ryukyuensis*, *P. sinensis*). The analysis was repeated using the percent fertilization data after 6–8 h. A further two-way ANOVA was carried out to compare the development of cleaved eggs through to blastulae in within- and between-morphospecies crosses. All of these analyses were done using both arcsine-transformed and raw data. Both methods produced similar results and only results from analyses of raw data are presented here.

A one-way ANOVA comparing fertilization rates after 2–3 h between morphospecies combinations was also done to determine whether there were significant differences in fertilization between morphological species-pair crosses. Replication was too low to compare all morphospecies-pair crosses; consequently this analysis was carried out only on crosses in which *P. daedalea* was the maternal colony, and for the sperm of *P. daedalea*, *P. lamellina*, *P. pini*, *P. ryukyuensis*, *P. sinensis* and selfs. The incidence of irregular and regular embryos was compared between crosses to determine whether irregular embryos were more common in between-morphospecies crosses.

Effect of sperm concentration on fertilization rates

To determine whether fertilization success was an artifact of using optimal sperm concentrations in experi-

Table I

Mean fertilization rates from fertilization trials between morphological species of Platygyra *based on all crosses tried over the $3\frac{1}{2}$-year study period*

	Eggs							
Sperm	PD	PH	PL	PP	PR	PS	PB	Selfs
PD	**45.2** (0–100) *n* = 49	18.3 (0–58.9) *n* = 5	66.8 (41–90) *n* = 13	65.4 (0–100) *n* = 11	36.3 (0–84.1) *n* = 8	52.6 (0–99.1) *n* = 18	1.3 (0–6.3) *n* = 2	3.1 (0–6) *n* = 28
PH	47.5 (0–94.2) *n* = 5	**51.5** (9–95.8) *n* = 2	—	24.6 (0–62.5) *n* = 4	—	77.1 (0–100) *n* = 2	—	1.4 (0–4.1) *n* = 2
PL	68.2 (1–100) *n* = 13	—	**58.1** (41–84) *n* = 6	—	63.8 (40–77) *n* = 2	72.3 (41–91) *n* = 2	—	2.7 (0–6.2) *n* = 4
PP	66.6 (6.5–99) *n* = 10	17.4 (0–41.8) *n* = 4	—	**58.6** (1–96.7) *n* = 4	—	77.4 (26–98) *n* = 2	—	8.9 (0–15) *n* = 5
PR	66.1 (7–100) *n* = 8	—	90.5 (90–92) *n* = 2	—	**57.7** (31–71) *n* = 2	70.6 (27–92) *n* = 4	—	6.2 (0–14.3) *n* = 2
PS	71.7 (0–100) *n* = 50	26.6 (13–44) *n* = 2	45.4 (0–90.8) *n* = 2	7.3 (0–27.7) *n* = 2	65.3 (7–100) *n* = 4	**50.1** (0–100) *n* = 8	—	3.7 (0–11) *n* = 6
PB	43.5 (31–57) *n* = 1	—	—	—	—	—	—	—

n = total number of crosses between morphospecies (*i.e.*, colony pairs). All crosses between colony pairs were replicated three times and the range of % fertilization over all replicates of all crosses is presented in parentheses. Conspecific crosses are in bold and crosses between morphospecies in normal type. '—' denotes cross not yet tried. 'Selfs' are crosses using eggs and sperm from the same colony. PD—*P. daedalea*, PL—*P. lamellina*, PP—*P. pini*, PR—*P. ryukyuensis*, PS—*P. sinensis*, PB—*Platygyra B*, PH—*Platygyra H*.

mental crosses, a sperm dilution series was carried out using two colonies each of *Platygyra daedalea* and *P. ryukyuensis* from Orpheus Island. Sperm was diluted to 10^5, 10^4, and 10^3 per ml and used in reciprocal crosses between all four colonies (within-morphospecies, between-morphospecies, selfs, and controls). Percent fertilization in all crosses was calculated after 2–3 h as described above.

Effect of egg age on fertilization rates

Due to the complex washing procedures and the nature of the experiments described above, it can be up to 6 h after spawning before eggs and sperm are combined in fertilization trials. Sperm kept in a concentrated form has been shown to age slowly (Chia and Bickell, 1983; Oliver and Babcock, 1992), although nothing is known about the effect of aging on the egg—particularly with regard to its ability to be fertilized with foreign sperm.

To test whether egg aging was influencing the results of fertilization trials, an experiment was carried out to look at fertilization rates in eggs of varying ages. Three colonies each of *P. daedalea* and *P. sinensis* from Magnetic Island were used. Once two colonies had spawned, gametes were washed as described above and immediately crossed with each other. As each successive colony spawned, gamete bundles were washed and immediately crossed with all other colonies that had already spawned. This was repeated until all six colonies had spawned, after which all crosses were repeated at 2-h intervals until eggs were 8-h old. All crosses were reciprocal and each cross was replicated twice at each time interval. Controls with sperm-free water were done at all stages of the experiment. Percent fertilization of within- and between-morphospecies crosses at various egg ages was compared for both *P. daedalea* and *P. sinensis*, and regression analysis was used to detect general trends associated with the effect of egg age on fertilization success for all cross types (within-morphospecies, between-morphospecies, self, and control).

Fertilization success as a function of genotype

Tissue samples from all colonies used in the fertilization trials in 1991 and 1992 were collected for genetic analysis to determine whether any genetic structuring, independent of morphology, was associated with breeding groups within the genus and whether incompatible colonies were clonemates. Colonies were screened using allozyme electrophoresis at nine polymorphic loci (PGM*, MPI*, CK*, LP*, LT-1*, LT-2*, LG-1*, LG-2*, and GPI*; see Miller, 1994b; Miller and Benzie, in press, for further details of electrophoretic techniques). The genetic difference between mated colonies, based on the nine-loci genotype, was calculated as a percentage based on the number of different alleles (*i.e.*, alleles not shared by the two colonies). The fertilization success between any two colonies and the genetic difference between them was then compared.

Larval rearing

In addition to the fertilization experiments described above, large numbers (>1000) of larvae from both within- and between-morphospecies crosses were reared and allowed to settle so that their subsequent development could be monitored through planulae to polyp. Larvae were raised using the methods described by Babcock and Heyward (1986). After 4–5 days, larvae were transferred from plastic jars (2–4 l) to closed aquaria in which settlement substrata (either coral rubble or terracotta paving tiles) were provided. Once all larvae had settled, settlement plates were transferred to a flow-through aquarium system where they were maintained to obtain specimens for examination of colony morphology.

Results

Fertilization trials

The fertilization trials among colonies of *Platygyra* showed that fertilization took place between all the morphological species tested (Table I). The gametes of the seven morphological species crossed were highly compatible, and mean fertilization rates among different morphospecies were similar (between 50% and 70%, Table I). In total, 389 crosses were conducted including 1140 separate vials. Although variability within replicate vials was sometimes high (Table II), the controls indicated no contamination of trials by foreign sperm. The high levels of fertilization (>50%) measured in crosses demonstrates that colonies were reproductively compatible. Interestingly, self-fertilization rates were low in all morphospecies (Table I).

Fertilization success was highly variable both among and within morphological species, with most differences occurring at the level of individual crosses. For example, fertilization success between any two *P. daedalea* colonies ranged from 9.8% to 67.1% in crosses carried out on 17 October 1992 (Table II) and between 0% and 81% between *P. sinensis* colonies on the same night (Table II). Similarly, the success of between-morphospecies crosses seems largely dependent on colony compatibility (Table II). No colony had consistently low fertilization with all other colonies (*e.g.*, Table II), so variability was not due simply to low overall viability of the gametes in any one colony.

Table II

Mean fertilization rates (± standard deviation) between three colonies of Platygyra daedalea *and three colonies of* P. sinensis *from fertilization trials carried out at Magnetic Island on 17 October 1992*

	Eggs					
Sperm	PD1	PD2	PD3	PS1	PS2	PS3
PD1	—	31.6 (0.0)	24.8 (14.07)	3.4 (1.84)	7.1 (3.82)	0.0 (0.00)
PD2	9.8 (0.21)	—	67.1 (3.96)	68.9 (0.0)	40.1 (3.18)	7.7 (1.98)
PD3	27.5 (6.51)	47.5 (15.63)	—	70.6 (1.13)	43.1 (15.91)	16.7 (23.55)
PS1	72.0 (5.66)	67.7 (1.34)	91.6 (4.74)	—	45.1 (3.04)	7.15 (10.11)
PS2	73.2 (3.25)	56.1 (6.79)	95.4 (0.0)	73.3 (2.69)	—	0.0 (0.00)
PS3	23.1 (11.24)	18.2 (4.31)	74.9 (0.71)	81.2 (2.05)	10.7 (7.00)	—

Self-fertilization rates (eggs and sperm from the same colony, denoted by "—") were <1% in all colonies except PD1 which was <3%. All controls (eggs in sperm-free water) had 0% fertilization.

In crosses for which *P. daedalea* was the maternal colony, ANOVA indicated significant variability in fertilization rates with different sperm types (df = 5, MS = 19043.31, $F = 25.65$, $P < 0.001$). However, the source of most of this variability lay in differences between rates of self fertilization rather than in crosses within and between morphological species. Tukey's HSD test showed no differences between fertilization rates for within-morphospecies crosses [PD × PD ($\bar{x} = 45.2$, $n = 49$)] and between-morphospecies crosses [PD × PS ($\bar{x} = 71.7$, $n = 50$), PD × PL ($\bar{x} = 68.2$, $n = 13$), PD × PP ($\bar{x} = 66.6$, $n = 10$), PD × PR ($\bar{x} = 66.1$, $n = 8$)]. These fertilization rates were, however, significantly different from rates of self fertilization in *P. daedalea* ($\bar{x} = 3.1$, $n = 28$).

Fertilization success between some morphological species was low. Crosses between *Platygyra* H eggs and *P. daedalea* sperm ($n = 5$ pairs), *Platygyra* H eggs and *P. pini* sperm ($n = 3$ pairs), *P. pini* eggs and *P. sinensis* sperm ($n = 2$ pairs) and *Platygyra* B eggs and *P. daedalea* sperm ($n = 1$ pair) had fertilization rates below 20% (Table I). However, the low fertilization rates between these morphospecies are within the range observed between colonies of the same morphospecies (Table I) and may not reflect species incompatibility. Larvae produced by crosses with low fertilization rates were regular in appearance and seemed to develop normally.

Reciprocal crosses between different morphological

Table III

Results from multiple two-way analyses of variance to compare fertilization success (in both within- and between-morphospecies crosses) for the seven different morphological species of Platygyra

Experimental data analyzed	Source of variation	df	*F*	Pr > *F*
2–3 h	fertilization type	1	0.08	0.77 (ns)
	egg species	6	1.64	0.13 (ns)
	fert-type × egg species	5	1.75	0.12 (ns)
6–8 h	fertilization type	1	0.53	0.46 (ns)
	egg species	6	2.20	0.04*
	fert-type × egg species	5	0.42	0.83 (ns)
Development	fertilization type	1	1.03	0.31 (ns)
	egg species	6	4.60	0.00***
	fert-type × egg species	5	0.59	0.71 (ns)

'Fertilization type' is within/between morphospecies crosses; 'egg species' is the morphological species from which eggs in crosses originated. Analyses were performed on data from both counts at 2–3 h and 6–8 h following fertilization, and to compare the development of embryos from both within- and between-morphospecies crosses. (ns)–not significant, * $P < 0.05$, *** $P < 0.001$.

species generally produced similar fertilization rates (*i.e.*, eggs and sperm from two different morphological species readily fertilized each other) (Table I). In some cases, however, compatibility in reciprocal crosses was asymmetrical. For example, *P. sinensis* eggs were readily fertilized by *P. pini* sperm (77.4% fertilization), whereas *P. pini* eggs were poorly fertilized by *P. sinensis* sperm (7.3% fertilization; Table I). A similar pattern existed between *Platygyra* B and *P. daedalea* (Table I), but replication was too low to determine if these differences were significant.

The overall rates of between-morphospecies crosses (all morphospecies pooled) were not significantly different from the rates of within-morphospecies fertilization ('fertilization type'—Table III; Fig. 1a, b), nor was there any difference in within- or between-species fertilizations among the different morphospecies ('fert-type × egg species'—Table III). These results indicate that fertilization can occur at the same rate both within and between morphological species of *Platygyra*.

There were no differences in mean fertilization rates in either within- or between-morphospecies crosses between 2–3 h and 6–8 h (Fig. 1a, b); therefore percent fertilization did not increase with egg-sperm contact time. About 80% of all eggs fertilized after 2–3 h had developed into blastulae after 6–8 h, and survival rates were the same in both within- and between-morphospecies crosses (Fig. 1c).

A comparison of regular *versus* irregular cleavages and embryos, at both 2–3 h and 6–8 h following fertilization, showed that the number of irregular cleavages was similar in both within- and between-morphospecies crosses and between 2–3 and 6–8 h (Fig. 2). Irregular embryos made up about 10%–15% of the 100 eggs counted (fertilized and unfertilized) after both 2–3 h and 6–8 h, whereas regular cleavages were, on average, 40%–50% of the 100 counted. The occurrence of irregular embryos does not appear to be related to differing morphology of

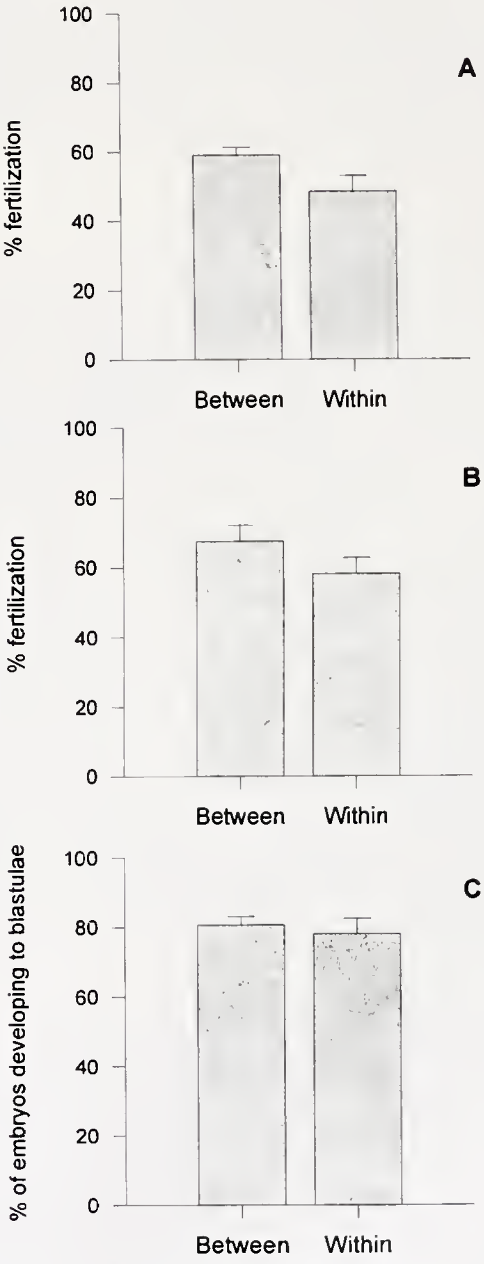

Figure 1. Fertilization success. Mean within- and between-morphospecies fertilization rates in fertilization trials between morphological species of *Platygyra*. Bars denote standard errors. (A) 2–3 h following gamete mixing; (B) 6–8 h following gamete mixing; (C) development of embryos after 6–8 h.

parent colonies but may be a consequence of the experimental method (see Oliver and Babcock, 1992).

Effects of sperm concentration on fertilization rates

There were no effects of sperm concentration on fertilization rates in either *P. daedalea* or *P. ryukyuensis* crosses. Fertilization rates for *P. daedalea* colonies fertilized with conspecific sperm did not differ significantly at sperm concentrations ranging from 10^3 to 10^5 per ml (Fig. 3). Similarly, sperm concentration did not affect fertilization rates in crosses of *P. daedalea* eggs with *P. ryukyuensis* sperm (Fig. 3) or in crosses of *P. ryukyuensis* eggs with conspecific sperm and with *P. daedalea* sperm (Fig. 3).

Effects of egg age on fertilization rates

The ability of an egg to be fertilized by sperm from a different morphological species does not appear to be a function of egg age. There was no significant change in within-morphospecies or between-morphospecies fertilization rates with egg age in either *P. daedalea* or *P. sinensis.* Regression analysis of percent fertilization with egg age was not significant for either *P. daedalea* or *P. sinensis* for any fertilization type (Fig. 4): within-morphospecies (r^2 = 0.005, 0.05 respectively), between-morphospecies (r^2 = 0.04, 0.1 respectively), self (r^2 = 0.07, 0.1 respectively), or controls (r^2 = 0.0004, 0.0 respectively). Although r^2 was not significant (possibly due to high variation in fertilization rates; see Fig. 4), fertilization (both within- and between-morphospecies) showed an overall decrease with egg age in *P. daedalea* and a general increase with egg age in *P. sinensis* (Fig. 4). Between-morphospecies fertilization rates were generally higher than within-morphospecies fertilization rates in *P. daedalea,* whereas between-morphospecies fertilization rates were lower than within-species fertilizations in *P. sinensis* (Fig. 4); however, neither difference was significant.

Fertilization success as a function of genotype

Fertilization success in colonies of *Platygyra* was not related to the genetic difference between parent colonies. No two colonies used in fertilization trials had identical nine-locus genotypes (*i.e.,* none were clonemates). Colonies that were between 40% and 60% different genetically had fertilization rates varying between 0% and 100% (Fig. 5). No evidence of what might be considered a self-recognition allele (Grosberg, 1988) was seen on the basis of the nine-locus genotypes of incompatible colonies. Colony pairs exhibiting low levels of fertilization did not possess consistent allelic or genotypic characteristics.

Temporal separation in spawning times

There was some evidence of temporal separation of spawning times between some morphological species of *Platygyra* (Fig. 6). However, this separation was not clearly defined and an overlap in spawning time existed between the different morphospecies. Although *Platygyra daedalea* colonies have been observed spawning throughout the night, *P. lamellina* and *P. pini* tend to spawn earlier in the evening (spawning 50 to 200 minutes after sunset), while *P. sinensis, P. ryukyuensis,* and *Platygyra* H generally spawn later (more than 200 minutes after sunset) (Fig. 3). The ranges of spawning times observed in the field and laboratory show that there was considerable overlap in spawning, and that the gametes of different morphological species were often released simultaneously.

Larval rearing

The embryos of within- and between-morphospecies crosses developed into ciliated planulae larvae within 2 days of fertilization and were competent to settle 4–5 days after spawning. Metamorphosed larvae from both fertilization types gained zooxanthellae within 2 days after settlement and subsequently began skeletal construction. After 5 months, recruits ranged in diameter from 2 to 10 mm. In March 1993 the oldest colony from a between-morphospecies cross had reached 3½ years of age, and colony diameters ranged between 2 and 5 cm. Unfortunately, all colonies died in 1993 because of a prob-

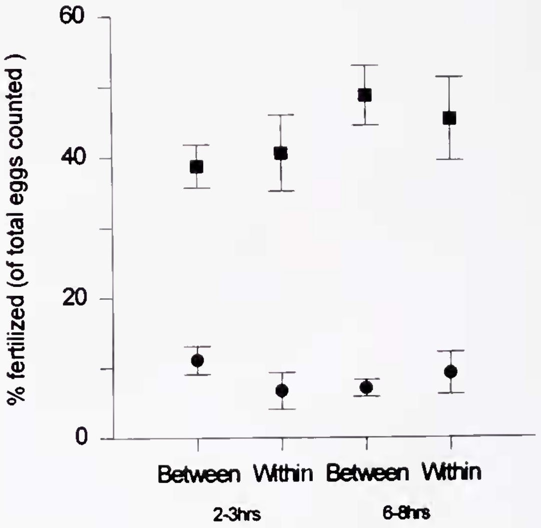

Figure 2. Embryo condition. Occurrence of regular and irregular embryos in both within- and between-morphospecies fertilization trials expressed as a percentage of the first 100 eggs counted per vial (all morphospecies pooled). Bars represent 95% confidence limits. ■—regular embryos; ●—irregular embryos.

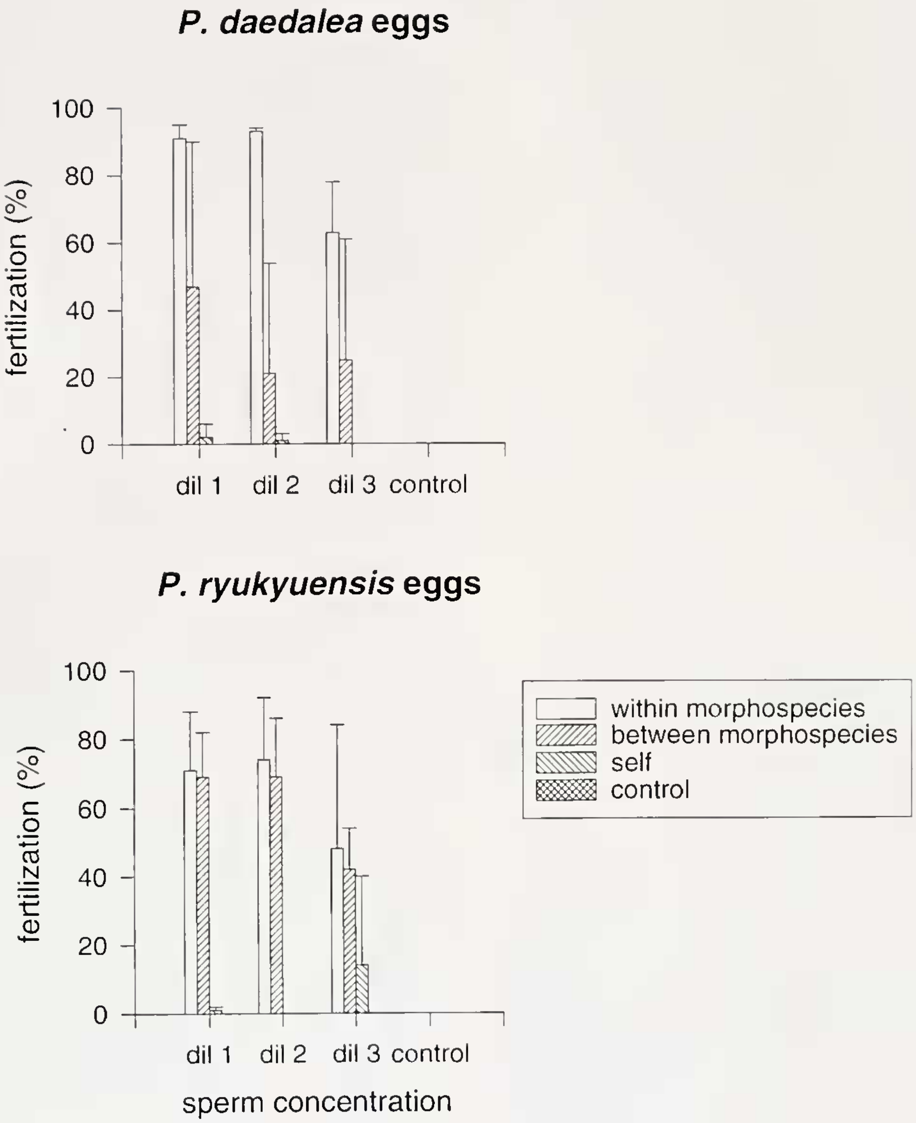

Figure 3. Effects of varying sperm concentration. Results from crosses between two colonies each of *Platygyra daedalea* and *P. ryukyuensis* in which sperm concentrations were varied 100-fold. Sperm concentrations: Dil 1 = 1×10^5 ml^{-1}, Dil 2 = 1×10^4 ml^{-1}, Dil 3 = 1×10^3 ml^{-1}. Error bars are standard deviations. Controls are with sperm-free water.

lem with the seawater system. Development of colonies from between-morphospecies crosses appeared to be normal up to that point, although their skeletal characteristics were not well enough developed to reveal whether they resembled any of the predefined morphological species or perhaps were intermediate in morphology between the parent colonies.

Discussion

Fertilization is clearly possible between the different morphological species of *Platygyra in vitro.* There appeared to be no relationship between morphology and reproductive compatibility since fertilization was recorded in all crosses. Fertilization occurred between most morphological *Platygyra* species at rates equivalent to within-species fertilizations (Table I, Fig. 1a, b). Nonsignificant differences in fertilization rate for comparisons among crosses in which *P. daedalea* was the maternal colony probably reflect widespread gametic compatibility within the genus. The low fertilization rates in some crosses between morphological species may reflect small sample sizes and differences in compatibility between individual colonies (Table III, Fig. 1a, b) rather than overall morphospecies incompatibility. Embryos resulting from between-morphospecies

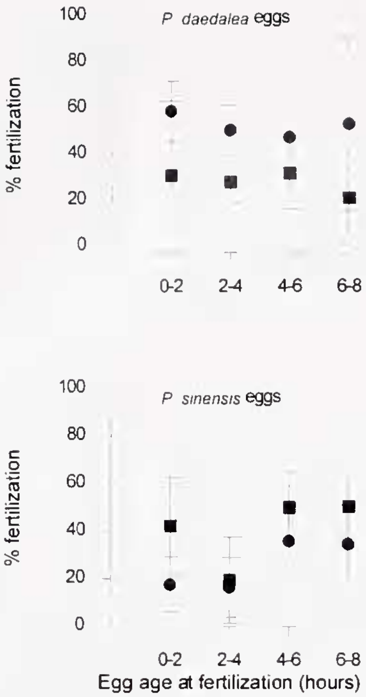

Figure 4. Effects of egg aging. Mean fertilization rates with increasing egg age in crosses between three colonies each of *Platygyra daedalea* and *P. sinensis*. Bars represent 95% confidence limits. ■—within-morphospecies crosses, ●—between-morphospecies crosses.

crosses apparently did not have reduced survivorship (Fig. 1c).

Percent fertilization was high after 2 h (the minimum time after fertilization in which cleavage becomes evident), and fertilization rates did not increase with egg-sperm contact time. This suggests that fertilization between gametes occurred promptly after the eggs were introduced into the vials, irrespective of the morphotype of the parent colonies. Variations in egg age, sperm dilution, and parental genotype failed to affect patterns of fertilization in crosses within or among morphospecies.

No evidence of pre-zygotic barriers to fertilization was detected among the morphological species of *Platygyra*. The seven morphological species have overlapping spawning times (Fig. 6), and high levels of fertilization in trials indicate that no species-specific gamete recognition systems (*e.g.*, Uehara *et al.*, 1990; Vacquier *et al.*, 1990; Palumbi, 1992) are operating in *Platygyra*. Preliminary investigations of sperm chemotaxis in *Platygyra* showed limited evidence of sperm attraction, but no evidence of species-specificity of sperm attraction in *Platygyra* (K. Miller and R. Miller, unpubl. data). Even if present, species-specific sperm chemotaxis could not be completely effective as a barrier to fertilization without membrane-level recognition or selectivity.

Mixed parentage does not appear to affect the development of *Platygyra* larvae or the competence of larvae to settle, metamorphose, and grow. Sterility of these offspring may be a post-zygotic isolating mechanism between morphological species of *Platygyra*, although costs associated with sterile progeny (*e.g.*, gamete wastage, loss of resources) may be high. However, in histological studies all mature-sized colonies of *P. sinensis* had gonads (Babcock, 1986) and in the 389 fertilization trials carried out, no colony was ever found that did not fertilize with at least one other colony, suggesting that sterile *Platygyra* are either rare or nonexistent.

Results from this study, combined with genetic studies (Miller and Benzie, in press), lead us to believe that fertilization and gene flow will occur between morphological "species" of *Platygyra*. This raises a number of perplexing questions. First, should these morphological groups within the genus *Platygyra* (Miller, 1994a) be considered true species, or is there simply one highly polymorphic species (*e.g.*, Skulason and Smith, 1995) within the genus? And second, if gene exchange occurs freely between all morphological types, how can the morphologically distinct groups be maintained in this genus?

Morphological species boundaries within the genus *Platygyra* are clear (Miller 1994a), and morphological differences appear to be far greater between *Platygyra* spp. than between other controversial species groups: for example, the three morphological variants of *Montastraea annularis* that are now considered sibling species (Knowlton *et al.*, 1992; Weil and Knowlton, 1994); the two morphs of *Montipora digitata* that have been identified as reproductively isolated species (Stobart and Benzie, 1994); and *Acropora cuneata* and *A. palifera*, which are difficult to distinguish by morphology but are genetically distinct (Ayre *et al.*, 1991). Hence it is not surprising that the seven morphological variants of *Platygyra* have been considered separate species in the past.

However, genetic and reproductive data indicate that *Platygyra* morphospecies are much more closely related than the *Montastrea* spp., the *Montipora* spp., or the two *Acropora* species. Furthermore, many groups of corals show discrepancies between species characters including morphology, genetics, and reproduction (Potts and Garthwaite, 1986; McMillan *et al.*, 1991; Willis *et al.*, 1992; Wallace and Willis, 1994; Romano and Palumbi, 1996). Defining species boundaries in corals is clearly a controversial issue. Nonetheless, our results show that in the case of *Platygyra*, it is inappropriate to consider the taxonomic unit as the minimal evolutionary unit and that evolutionary processes in different morphological species of *Platygyra* will be linked.

The maintenance of seven morphologically distinct entities in *Platygyra* in spite of genetic interchange poses a challenging question. Progeny from between-morphospecies crosses may be expected to have morphological

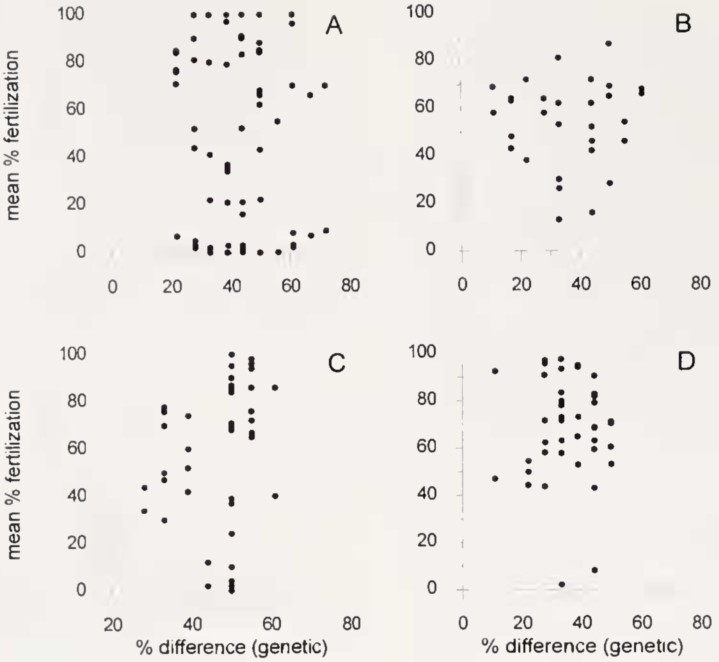

Figure 5. Fertilization success as a function of genotype. Comparison of percent fertilization (based on the mean of three replicates) and allelic differences between colonies used in fertilization trials. (A) Magnetic Island, 1991; (B) Magnetic Island, 1992; (C) Davies Reef, 1991; (D) Orpheus Island, 1992.

characters intermediate between those of the parent colonies and, with continued interbreeding, distinctions between the morphological species would be likely to break down. However, nothing is known of the mechanisms that produce skeletal differences in corals; if morphology is linked to dominance alleles, then between-morphospecies progeny may display the skeletal characteristics of only one parent (*e.g.*, Strathmann, 1981; Byrne and Anderson, 1994). Alternatively, processes such as introgression may play a role in the maintenance of morphological differences in *Platygyra* (Miller and Benzie, in press).

Reproductive and distributional variation may also provide some level of segregation between the morphospecies, thus helping to maintain morphological differences. There is some separation among spawning times of *Platygyra* morphospecies on the Great Barrier Reef, but these do not constitute complete temporal barriers to fertilization (Fig. 6). Nevertheless, most egg-sperm interactions take place in the first few seconds after mature eggs are in contact with a sperm suspension (Fig. 1a; Denny and Shibata, 1989; Mundy *et al.*, 1994), and colonies are more likely to be fertilized by close neighbors (Oliver and Babcock, 1992). These factors in combination could result in a degree of temporal reproductive segregation, if not total isolation.

Sophisticated egg-sperm recognition systems are clearly operational at several levels in *Platygyra*. Colonies of *Platygyra* do not frequently self-fertilize (Table I), unlike *Goniastrea favulus*, which commonly self-fertilizes (Heyward and Babcock, 1986; Stoddart *et al.*, 1988). In addition, the high variability in fertilization rates both within and between morphological species also indicates some level of incompatibility or individual recognition between certain pairs of individuals. This degree of reproductive separation in combination with small differences in the timing of spawning is probably insufficient to produce fixed allelic differences in the populations, but it may produce the indications of nonrandom mating present in the genotypic structure of *Platygyra* populations (Miller and Benzie, in press).

No clear-cut habitat separation is apparent in *Platygyra*, where all morphospecies can be found in various habitats on a single reef (Miller, 1994a). At large scales, however, trends in the distribution of morphospecies are apparent. For example, *P. lamellina* is rare on mid-shelf and shelf-edge reefs in the central Great Barrier Reef; *P. pini* is rare on coastal reefs; *P. ryukyuensis* is most common on coastal fringing reef flats; and *P. lamellina*, *P. ryukyuensis*, and *Platygyra* H are rare or absent on the reefs from the southern Great Barrier Reef (Miller, 1994b). Across even larger scales, further discontinuities in species distributions are evident (Veron, 1993), and observations of spawning times of *Platygyra* in other regions (*e.g.*, Okinawa: Heyward *et al.*, 1987; Hayashibara *et al.*, 1993) indicate that on geographical scales segregation of spawning times among *Platygyra*

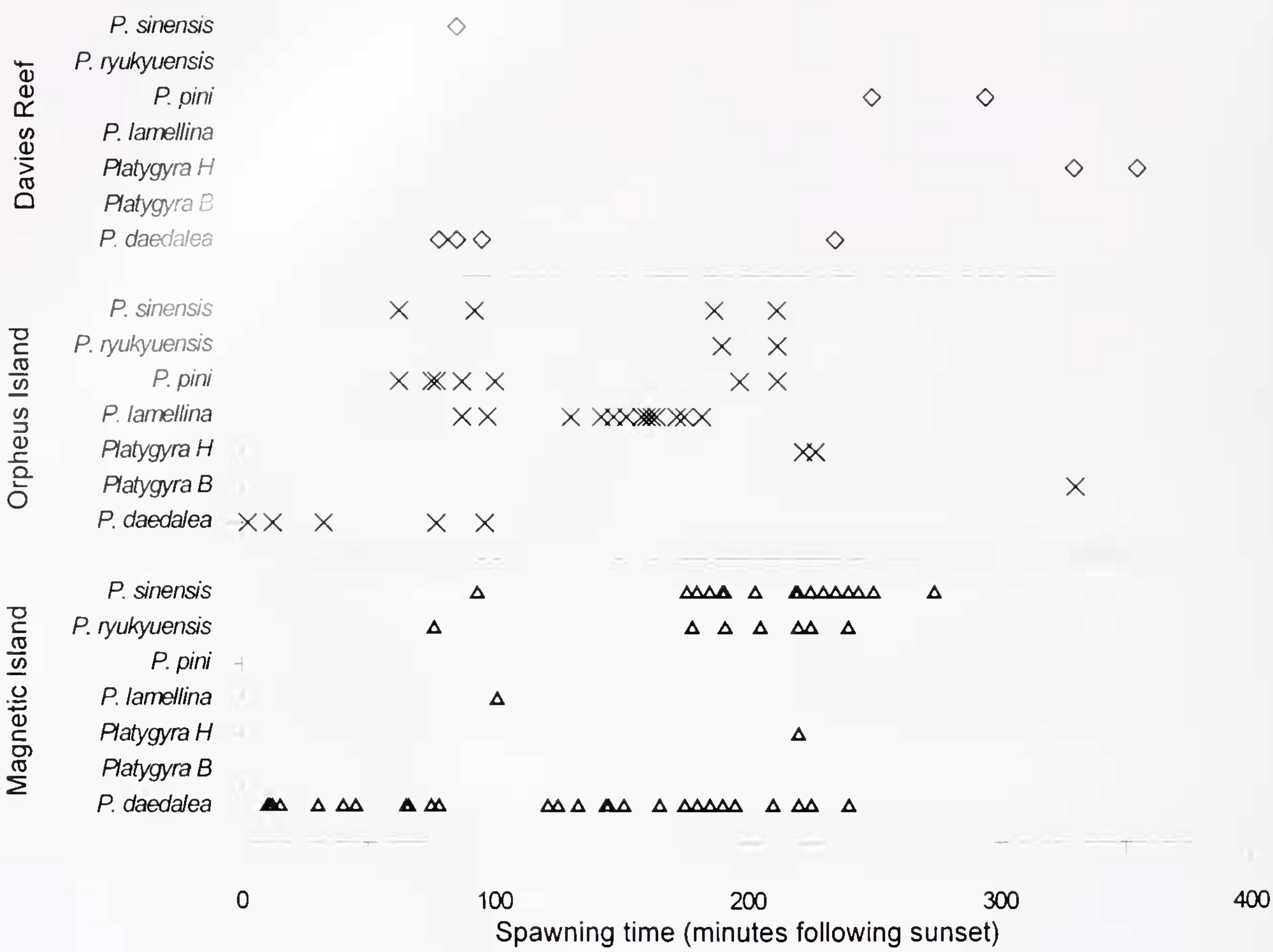

Figure 6. Temporal separation in spawning. Spawning times of *Platygyra* colonies at three reefs on the Great Barrier Reef. Spawning times include observations made by scuba divers *in situ*, spawning times of experimental colonies in aquaria (these have been shown not to differ from *in situ* spawning times, see Miller, 1994b), and spawning times recorded by Babcock *et al.* (1986).

morphospecies could result in reproductive isolation. The distribution of the morphological species of *Platygyra* clearly exhibits some habitat-level differences that may contribute to the continued coexistence of the morphospecies, specifically through differential fitness in various habitats and the subsequent spatial and temporal reproductive isolation in certain habitats.

It has recently been proposed that reticulate evolution occurs within the Scleractinia (Veron, 1995). *Platygyra* morphospecies are widespread throughout the Indo-Pacific (Veron, 1993) and, as we have discussed, there are varied levels of differentiation and connectedness between the morphological or taxonomic units. Surface-circulation vicariance mechanisms (Veron, 1995) and reticulate evolution may well be the basis for the morphological and genetic variation in *Platygyra* populations across both local and geographic scales, and the likelihood of this linkage among morphological species of *Platygyra* should be accepted.

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Literature Cited

Ayre, D. J., J. E. N. Veron, and S. L. Dufty. 1991. The corals *Acropora palifera* and *Acropora cuneata* are genetically and ecologically distinct. *Coral Reefs* **10:** 13–18.

Babcock, R. C. 1986. The population ecology of reef flat corals of the family Faviidae (*Goniastrea, Platygyra*). Doctor of Philosophy Thesis, James Cook University, Townsville. 163 pp.

Babcock, R. C., and A. J. Heyward. 1986. Larval development of certain gamete spawning scleractinian corals. *Coral Reefs* **5:** 111–116.

Babcock, R. C., G. D. Bull, P. L. Harrison, A. J. Heyward, J. K. Oliver, C. C. Wallace, and B. L. Willis. 1986. Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef. *Mar. Biol.* **90:** 379–394.

Byrne, M., and M. J. Anderson. 1994. Hybridization of sympatric *Patiriella* species (Echinodermata: Asteroidea) in New South Wales. *Evolution* **48:** 549–577.

Chevalier, J. P. 1975. *Les scleractinaires de la Melanesie Francaise (Nouvelle Caledonie, Iles Chesterfield, Iles Loyaute, Nouvelles Hebrides).* 2eme Partie. Exped. Francaise recifs corallens Nouvelle Caledonie. Edn. Fond. Singer-Polignac, Paris. 507 pp.

Chia, F. S., and L. R. Bickell. 1983. Echinodermata. Pp. 545–620 in *Reproductive Biology of Invertebrates,* Vol. 2: Spermiogenesis and sperm function. John Wiley & Sons, New York.

Coll, J. C., B. F. Bowden, G. V. Meehan, G. M. Konig, A. R. Carroll, D. M. Tapiolas, P. M. Alino, A. Heaton, R. De Nys, P. A. Leone, M. Maida, T. L. Aceret, R. H. Willis, R. C. Babcock, B. L. Willis, Z. Florian, M. N. Clayton, and R. L. Miller. 1994. Chemical aspects of mass spawning in corals. I. Sperm attractant molecules in the eggs of the scleractinian coral *Montipora digitata. Mar. Biol.* **118:** 177–182.

Darwin, C. 1859. *On the Origin of Species.* John Murray, London. 513 pp.

Denny, M. W., and M. F. Shibata. 1989. Consequences of surf zone turbulence for settlement and external fertilization. *Am. Nat.* **134:** 859–889.

Ellis, J., and D. Solander. 1786. *The Natural History of Many Curious and Uncommon Zoophytes.* Benjamin White and Peter Elmsly, London. 208 pp.

Grosberg, R. K. 1988. The evolution of allorecognition specificity in clonal invertebrates. *Q. Rev. Biol.* **63:** 377–412.

Harrison, P. L., R. C. Babcock, G. D. Bull, J. K. Oliver, C. C. Wallace, and B. L. Willis. 1984. Mass spawning in tropical reef corals. *Science* **223:** 1186–1189.

Hayashibara, T., K. Shimoike, T. Kimura, S. Hosaka, A. Heyward, P. Harrison, K. Kudo, and M. Omori. 1993. Patterns of coral spawning at Akajima Island, Okinawa, Japan. *Mar. Ecol. Prog. Ser.* **101:** 253–262.

Heyward, A. J., and R. C. Babcock. 1986. Self- and cross-fertilization in scleractinian corals. *Mar. Biol.* **90:** 191–195.

Heyward, A., K. Yamazato, T. Yeemin, and M. Minei. 1987. Sexual reproduction of corals in Okinawa. *Galaxea* **6:** 331–343.

Hodgson, G. 1988. Potential gamete wastage in synchronously spawning corals due to hybrid inviability. *Proc. 6th Int. Coral Reef Symp.* **2:** 707–714.

Knowlton, N., E. Weil, L. A. Weight, and H. M. Guzman. 1992. Sibling species in *Montastrea annularis,* coral bleaching, and the coral climate record. *Science* **255:** 330–333.

Lang, J. C. 1984. Whatever works: the variable importance of skeletal and of non-skeletal characters in scleractinian taxonomy. *Palaeontogr. Am.* **54:** 18–44.

Mayr, E. 1942. *Systematics and the Origin of Species.* Dover publications Inc., New York. 334 pp.

McMillan, J., T. Mahoney, J. E. N. Veron, and D. J. Miller. 1991. Nucleotide sequencing of highly repetitive DNA from seven species in the coral genus *Acropora* (Cnidaria: Scleractinia) implies a division contrary to morphological criteria. *Mar. Biol.* **110:** 323–327.

Metz, E. C., and S. R. Palumbi. 1996. Positive selection and sequence rearrangements generate extensive polymorphisms in the gamete recognition protein bindin. *Mol. Biol. Evol.* **13:** 397–406.

Miller, K. J. 1994a. Morphological species boundaries in the coral genus *Platygyra:* environmental variation and taxonomic implications. *Mar. Ecol. Prog. Ser.* **110:** 19–28.

Miller, K. J. 1994b. The *Platygyra* species complex: implications for coral taxonomy and evolution. Ph.D. Dissertation, James Cook University of North Queensland. 164 pp.

Miller, K. J., and J. A. H. Benzie. In press. No clear genetic distinction between morphological species within the coral genus *Platygyra. Bull. Mar. Sci.* **60:** ???

Miller, R. 1979. Sperm chemotaxis in the hydromedusae. 1. Species specificity and sperm behaviour. *Mar. Biol.* **53:** 99–114.

Miller, R. 1985. Demonstration of sperm chemotaxis in the Echinodermata: Holothuroidea, Ophiuroidea. *J. Exp. Zool.* **234:** 383–414.

Mundy, C., R. Babcock, I. Ashworth, and J. Small. 1994. A portable, discrete-sampling submersible plankton pump and its use in sampling starfish eggs. *Biol. Bull.* **186:** 168–171.

Oliver, J., and R. Babcock. 1992. Aspects of the fertilization ecology of broadcast spawning corals: sperm dilution effects and *in situ* measurements of fertilization. *Biol. Bull.* **183:** 409–417.

Palumbi, S. R. 1992. Marine speciation on a small planet. *TREE* **7:** 114–118.

Potts, D. C., and R. L. Garthwaite. 1986. Population genetics of the genus *Porites. Abstr. Annu. Meet. Int. Soc. Reef Stud.* p. 38.

Romano, S. L., and S. R. Palumbi. 1996. Evolution of Scleractinian corals inferred from molecular systematics. *Science* **271:** 640–642.

Skulason, S., and T. B. Smith. 1995. Resource polymorphisms in vertebrates. *TREE* **10:** 366–370.

Stobart, B., and J. A. H. Benzie. 1994. Allozyme electrophoresis demonstrates that the scleractinian coral *Montipora digitata* is two species. *Mar. Biol.* **118(2):** 183–190.

Stoddart, J. A., R. C. Babcock, and A. J. Heyward. 1988. Self-fertilization and maternal enzymes in the planulae of the coral *Goniastrea favulus. Mar. Biol.* **99:** 489–494.

Strathmann, R. R. 1981. On barriers to hybridization between *Strongylocentrotus drobachiensis* (O. F. Muller) and *S. pallidus* (G. O. Sars). *J. Exp. Mar. Biol. Ecol.* **55:** 39–47.

Uehara, T., H. Asakura, and Y. Arakaki. 1990. Fertilisation blockage and hybridisation among species of sea urchins. Pp. 305–310 in *Advances in Invertebrate Reproduction 5.* Elsevier Science, Amsterdam.

Vacquier, V. D., K. R. Carner, and C. D. Stout. 1990. Species-specific sequences of abalone lysin, the sperm protein that creates a hole in the egg envelope. *Proc. Natl. Acad. Sci. USA* **87:** 5792–5796.

Van Veghel, M. L. J. 1994. Reproductive characteristics of the polymorphic Caribbean reef building coral *Montastrea annularis.* I. Gametogenesis and spawning behavior. *Mar. Ecol. Prog. Ser.* **109:** 209–219.

Van Veghel, M. L. J., and R. P. M. Bak. 1993. Intraspecific variation of a dominant Caribbean reef building coral, *Montastrea annularis:* genetic behavioural and morphometric aspects. *Mar. Ecol. Prog. Ser.* **92:** 255–265.

Vaughan, T. W., and J. W. Wells. 1943. Revision of the sub-orders, families and genera of the Scleractinia. *Geol. Soc. Am. Spec. Pap.* **44:** 1–363.

Veron, J. E. N. 1993. Biogeography of hermatypic corals. *Australian Institute of Marine Science Monograph Series Volume 10,* Townsville. 433 pp.

Veron, J. E. N. 1995. *Corals in Space and Time.* University of New South Wales Press, Sydney. 321 pp.

Veron, J. E. N., M. Pichon, and M. Wijsman-Best. 1977. Scleractinia of eastern Australia, Part 2, Families Faviidae, Trachyphyllidae. *Australian Institute of Marine Science Monograph Series Volume 3*, Townsville. 233 pp.

Wallace, C. C., and B. L. Willis. 1994. The systematics of the coral genus *Acropora*: implications of the new biological findings for species concepts. *Annu. Rev. Ecol. Syst.* **25**: 237–262.

Weil, E., and N. Knowlton. 1994. A multi-character analysis of the Caribbean coral *Montastaea annularis* (Ellis and Solander, 1786) and its two sibling species, *M. faveolata* (Ellis and Solander, 1786) and *M. franksi* (Gregory, 1895). *Bull. Mar. Sci.* **55**: 151–175.

Willis, B. L. 1990. Species concepts in extant scleractinian corals: considerations based on reproductive biology and genotypic population structures. *Syst. Bot.* **15**: 136–149.

Willis, B. L., R. C. Babcock, P. L. Harrison, J. K. Oliver, and C. C. Wallace. 1985. Patterns in the mass spawning of corals on the Great Barrier Reef from 1981 to 1984. *Proc. 5th Int. Coral Reef Symp.* **4**: 343–348.

Willis, B. L., R. C. Babcock, P. L. Harrison, and C. C. Wallace. 1992. Experimental evidence of hybridisation in reef corals involved in mass spawning events. *Proc. 7th Int. Coral Reef Symp.* **Abstracts**: 109.